PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY
(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US2005/012270	12.04.2005	15.04.2004

International Patent Classification (IPC) or both national classification and IPC
INV. C07K19/00 A61P35/00 A61K31/195
ADD. C07K16/30 C07K14/245 C12N9/86

Applicant
GENENCOR INTERNATIONAL, INC.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☒ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☒ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:	Date of completion of this opinion	Authorized Officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	see form PCT/ISA/210	Wagner, R Telephone No. +49 89 2399-7357

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2005/012270

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed

   ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1 (b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ on paper

   ☒ in electronic form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed.

   ☐ filed together with the international application in electronic form.

   ☒ furnished subsequently to this Authority for the purposes of search.

3. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

Box No. II Priority

1. ☐ The validity of the priority claim has not been considered because the International Searching Authority does not have in its possession a copy of the earlier application whose priority has been claimed or, where required, a translation of that earlier application. This opinion has nevertheless been established on the assumption that the relevant date (Rules 43*bis*.1 and 64.1) is the claimed priority date.

2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43*bis*.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

   see separate sheet

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of

☐ the entire international application

☒ claims Nos. 15-34 (IA)

because:

☒ the said international application, or the said claims Nos. 15-34 (regarding IA) relate to the following subject matter which does not require an international search *(specify)*:

see separate sheet

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed *(specify)*:

☐ no international search report has been established for the whole application or for said claims Nos.

☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit:

- ☐ furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.
- ☐ furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.
- ☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rules 13*ter*.1(a) or (b).

☐ a meaningful opinion could not be formed without the tables related to the sequence listings; the applicant did not, within the prescribed time limit, furnish such tables in electronic form complying with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions, and such tables were not available to the International Searching Authority in a form and manner acceptable to it.

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in electronic form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See Supplemental Box for further details

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2005/012270

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-34
	No:	Claims	
Inventive step (IS)	Yes:	Claims	7,14,21,
	No:	Claims	1-6,8-13,15-20,22-34
Industrial applicability (IA)	Yes:	Claims	1-14
	No:	Claims	

2. Citations and explanations

see separate sheet

Box No. VI Certain documents cited

1. Certain published documents (Rules 43*bis*.1 and 70.10)

and / or

2. Non-written disclosures (Rules 43*bis*.1 and 70.9)

see form 210

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY (SEPARATE SHEET)

International application No.
PCT/US2005/012270

Re Item I
Basis of the report

The sequence listing (pages 1-20) filed on 14.12.2005 with the letter dated 12.12.2005 does not form part of the application as filed

Re Item II
Priority
It appears that the priority USP 562386 (15.04.2004) validly claims the claimed CAB molecules.

Re Item III
Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claims 15-34 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(I) PCT).

Re Item V
Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Reference is made to the following documents:
   D1: MEYER D L ET AL: "SITE-SPECIFIC PRODRUG ACTIVATION BY ANTIBODY--LACTAMASE CONJUGATES: PRECLINICAL INVESTIGATION OF THE EFFICACY AND TOXICITY OF DOXOXRUBICIN DELIVERED BY ANTIBODY DIRECTED CATALYSIS" BIOCONJUGATE CHEMISTRY, ACS, WASHINGTON, DC, US, vol. 6, no. 4, 1 July 1995 (1995-07-01), pages 440-446, XP000517229 ISSN: 1043-1802
   D2: WO 03/055527 A (VLAAMS INTERUNIVERSITAIR INSTITUUT VOOR BIOTECHNOLOGIE VZW; REVETS, HI) 10 July 2003 (2003-07-10)

D3: WU A M ET AL: "Tumor localization of anti-CEA single-chain Fvs: improved targeting by non-covalent dimers" IMMUNOTECHNOLOGY, ELSEVIER SCIENCE PUBLISHERS BV, NL, vol. 2, no. 1, February 1996 (1996-02), pages 21-36, XP004052689 ISSN: 1380-2933

D4: MCDONAGH CHARLOTTE F ET AL: "Improved yield and stability of L49-sFv-beta-lactamase, a single-chain antibody fusion protein for anticancer prodrug activation, by protein engineering." BIOCONJUGATE CHEMISTRY, vol. 14, no. 5, 25 July 2003 (2003-07-25), pages 860-869, XP002378783 ISSN: 1043-1802

D5: STICKLER M M ET AL: "CD4+ T-CELL EPITOPE DETERMINATION USING UNEXPOSED HUMAN DONOR PERIPHERAL BLOOD MONONUCLEAR CELLS" JOURNAL OF IMMUNOTHERAPY, LIPPINCOTT WILLIAMS & WILKINS, HAGERSTOWN, MD, US, vol. 23, no. 6, November 2000 (2000-11), pages 654-660, XP008040520 ISSN: 1524-9557

2. Claim 1 is directed to an ADEPT (antibody directed enzyme pro-drug therapy) compound, i.e. a single-chain antibody directed against CEA coupled to a ß-lactamase, defined by SEQ ID NO: 2. Said construct is not disclosed in the available prior art, thus the subject-matter of claim 1 is novel (Article 33(3) PCT)
The prior art (D1, page 441, second paragraph) discloses a construct between an anti-CEA Fab and a ß-lactamase as well as a construct between a single domain antibody against CEA and a ß-lactamase (D2, example 1). In both cases the constructs are intended to be used in the context of an ADEPT method for the treatment of cancer.

The difference between the disclosure of the prior art consists in the provision of an alternative targeting moiety. In view of the fact that the single-chain antibody T84.66 is known to have a high binding affinity (D3, table 1), the choice of the said single chain anti-CEA is an obvious alternative, which does not confer any surprising effects to the construct. Therefore the subject-matter of claim 1 does not involve an inventive step (Article 33(3) PCT).

3. Independent claims 2, 3 and 4 are further characterised by an amino acid position in

which a modification has occurred. Said features are not considered to be limiting because the skilled person, having a final product in his hands, cannot determine whether it falls within the scope of said claims or not. Therefore said features are not able to confer an inventive step on the subject-matter of claims 2,3 and 4 (Article 33(3) PCT).

4. In dependent claim 6 the single-chain antibody, originating from antibody 84.66 directed against CEA and coupled to a ß-lactamase is modified by two amino acid substitutions in the framework regions of the single chain antibody. These amino acid changes have been introduced by combinatorial consensus mutagenesis (CCM) to increase the expression of the construct in E. Coli. As CCM is commonly used to improve the expression of proteins in general and scFv-ß lactamase constructs in particular, see D4, page 862, the amino acid modifications of claim 6 do not confer an inventive step on construct (Article 33(3) PCT).

5. In dependent claim 7 a further two amino acid substitutions in position K283A and S586A are introduced in the ß-lactamase moiety. Said substitutions were introduced with the aim to reduce the immunogenicity of the bacterial ß-lactamase in the construct. As it is a general goal or pharmaceutical research to develop compounds for human treatment with the least possible immunogenicity in humans, the skilled person will use the known method of identifying the $CD4^+$ T cell determinants (see D5) of the enzyme which react with the $CD4^+$ T cells of common human donors and determine by a common alanine-scan the relevant amino acids. Therefore the two substitutions in position K283A and S586A, per se, do not confer an inventive step on the construct. As it was, however, not predictable that amino acid substitutions, which reduce the immunogenicity of b-lactamase would not reduce the expression in E. Coli or interfere with the folding of the construct, the subject-matter of claim 7 is considered to involve an inventive step (Article 33(3) PCT).

6. As claims 8-14 are directed to nucleic acids encoding exactly the same CAB constructs as those defined in claims 1-7, the same objections apply to claims 8-14, i.e claims 8-13 do not involve an inventive step, whereas claim 14 does involve an inventive step (Article 33(3) PCT).

7. As the ADEPT method of D1 is intended for medical use in humans, the method of treatment of claims 15-20, using the non-inventive CAB molecules (as defined also in claims 1-6, see above) does not involve an inventive step (Article 33(3) PCT).
The method of treatment using the novel and inventive CAB molecule of claim 7, is also new (Article 33(2) PCT) and involves an inventive step (Article 33(3) PCT).

The dosing schedules of claims 22-32, which are dependent on claim 15, do not confer an inventive step on the method because it is a general step of drug development to determine the optimal dosing schedule of an ADEPT method.

The melphalin-based pro-drug GC-Mel is well known in the field as an anticancer pro-drug, which is cleavable by a ß-lactamase. Thus the subject-matter of claims 33 and 34 does not involve an inventive step (Article 33(3) PCT).

8. For the assessment of the present claims 15-34 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

9. Further Remarks:

The abbreviation CAB is not a commonly used acronym and thus not clear (Article 6 PCT). It's meaning should have been indicated at least in the first claim in which it appears. The same applies to CAB 1.11, which is an internal denomination and therefore not clear (Article 6 PCT).
Claims 2-7, 9-14 are not clear (Article 6 PCT) because the positions of amino-acid modifications are not directly relating to the positions in a specific molecule.

Re Item VI

Certain documents cited

Certain published documents (Rule 70.10)

Application No Patent No	Publication date *(day/month/year)*	Filing date *(day/month/year)*	Priority date *(valid claim)* *(day/month/year)*
WO2005/058236	30.06.2005	10.12.2004	12.12.2003

is considered as not being part of the prior art (Rule 64.1 PCT). However this document may be of importance regarding novelty in a subsequent national/regional phase.